PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:

Mouritsen et al. Group Art Unit:

Serial No.: 08/809,314 Examiner: Schwadron

Filed: October 21, 1997

For: INDUCING ANTIBODY RESPONSE AGAINST SELF-PROTEINS WITH THE AID OF FOREIGN T-CELL EPITOPES

DECLARATION OF PROFESSOR SVEN FRØKJAER

I, Professor Sven Frøkjaer, PhD., residing at Solvej 6, 2840 Holte, Denmark,

hereby declare as follows:

1. My *Curriculum Vitae* is attached.

2. I have reviewed the contents of the above-captioned application. Based upon my review of the captioned application, it is my understanding that the present invention is directed to the surprising discovery that injecting recombinant self-proteins (self-protein analogs) having a preserved tertiary structure, the proteins having one or more of its peptide fragments substituted with a corresponding number of foreign T cell epitopes, induces an auto-antibody response against unmodified self-proteins.

3. As discussed in the patent application at page 4, line 4, it is important to preserve the overall tertiary structure of the original self-protein in order to optimize its therapeutic effect. Indeed a change in tertiary structure of a self-protein would increase the risk of inducing antibody responses to sequentially native regions of the protein now having a changed structure, and not only to remaining native and amino acid sequence modified regions.

4. It is my understanding, as a person of skill in the art of protein and peptide formulation, that the self- protein analog, having a preserved tertiary structure, can be prepared by selecting peptides comprising appropriate immunodominant epitopes, exchanging peptides sequences of essentially the same length in various parts of the self-protein molecule and determining the raised antibody response by suitable assay techniques.

5. In order to ascertain whether the tertiary structure of the self-protein has been preserved, thereby obtaining optimal therapeutic effects, screening procedures would be necessary. Such screening procedures would be routinely part of a drug development program's search for lead compounds and would be considered early on in the development phase to evaluate which modified self-proteins or self-protein analogs have preserved the tertiary

structure of the original protein. Such procedures involve standard experimental techniques for which there are numerous publications. Reference is made to one in particular, which is used as a text book, on protein characterization, e.g., fluorescence spectroscopy, near U.V. circular dichroism, Fourier transformed infrared spectroscopy and multi-dimensional NMR techniques, namely, "Physical Methods to Characterize Pharmaceutical Proteins", *Pharmaceutical Biotechnology*, Vol.7, Eds., J.N. Heron, W. Jisjoot & D.J.A. Crommelin, Plenum Press, New York, (1995). Ideally, for a screening process for lead compounds, two or more of the above techniques would be carried out in order to evaluate whether there is a change in the tertiary structure of the protein.

6. In conclusion, I consider screening for changes in tertiary structure to be a straightforward procedure that any company involved in drug development of protein-based drugs should be able to perform routinely as an integral part of the development process.

7. Furthermore, I believe that the preparation of a vaccine containing such a self-protein analog would be easily formulated based on my knowledge of protein and peptide drug formulations and delivery systems and on the guidance provided in the specification

of the above-captioned application. Strategies for developing and administering vaccines are generally well known in the art.

8. Formulation of vaccines based on purified proteins, such as the modified self-proteins of the present invention, generally correspond to formulation strategies for any other protein-based drug product. Potential problems and the guidance required to overcome problems connected therewith - as for instance preservation of tertiary structure - are dealt with in several textbooks, e.g., *Therapeutic Peptides and Protein Formulation, Processing and Delivery Systems*, Ed. A.K. Banga; Technomic Publishing AG, Basel 1995. The use of an adjuvant, e.g., aluminum hydroxide, aluminum phosphate (Adju-Phos), calcium phosphate, muramyl dipeptide analog, or some of the more recent developments in vaccine adjuvants such as biodegradable microparticles and Iscoms is a formulation challenge familiar to a pharmaceutical scientist working in this area. Likewise, the use of pharmaceutically acceptable excipients such as, e.g., water, sucrose, glycerol and sodium chloride, is merely routine to a person skilled in the art.

9. Vaccines are conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Other modes of administration such as nasal and oral formulations

may also be considered. In general, vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective and immunogenic. The amount of active ingredient is optimized, by routine experimentation.

10. I declare further that all statements made herein of my own knowledge are true and that all statements made or information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

1998.05.07
Date

Professor Sven Frøkjaer

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